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05 June 2002

Ms. Christie Whitman, Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Re: **Chemical Right-to Know HPV Challenge Program Submission**
HPV registration number

Dear Ms. Whitman:

Cardolite Corporation, Inc. is pleased to submit the enclosed test plan and robust summaries for Cashew Nut Shell Liquid (CAS RN 8007 -24-7) under the HPV Challenge Program, AR-201. Cardolite Corporation, Inc. is submitting this information directly to EPA with the understanding that a 120- day review period for public comment will follow this submission. The test plan and robust summaries are being submitted in electronic format as MS Word documents.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Chris Ford
Q.A. Manager
Cardolite Corporation

c: Tony Stonis – Cardolite Corporation

AR201-13793A

HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For

CASHEW NUT SHELL LIQUID

CAS No. 8007-24-7

Submitted to the US EPA

By

Cardolite Corporation, Inc.
www.cardolite.com

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Test Plan for Cashew Nut Shell Liquid

1. Introduction

Cashew nut shell liquid (CNSL) is one of the sources of naturally occurring phenols. It is obtained from the shell of a cashew nut. About 30-35% CNSL is present in the shell, which amounts to approximately 67% of the nut.

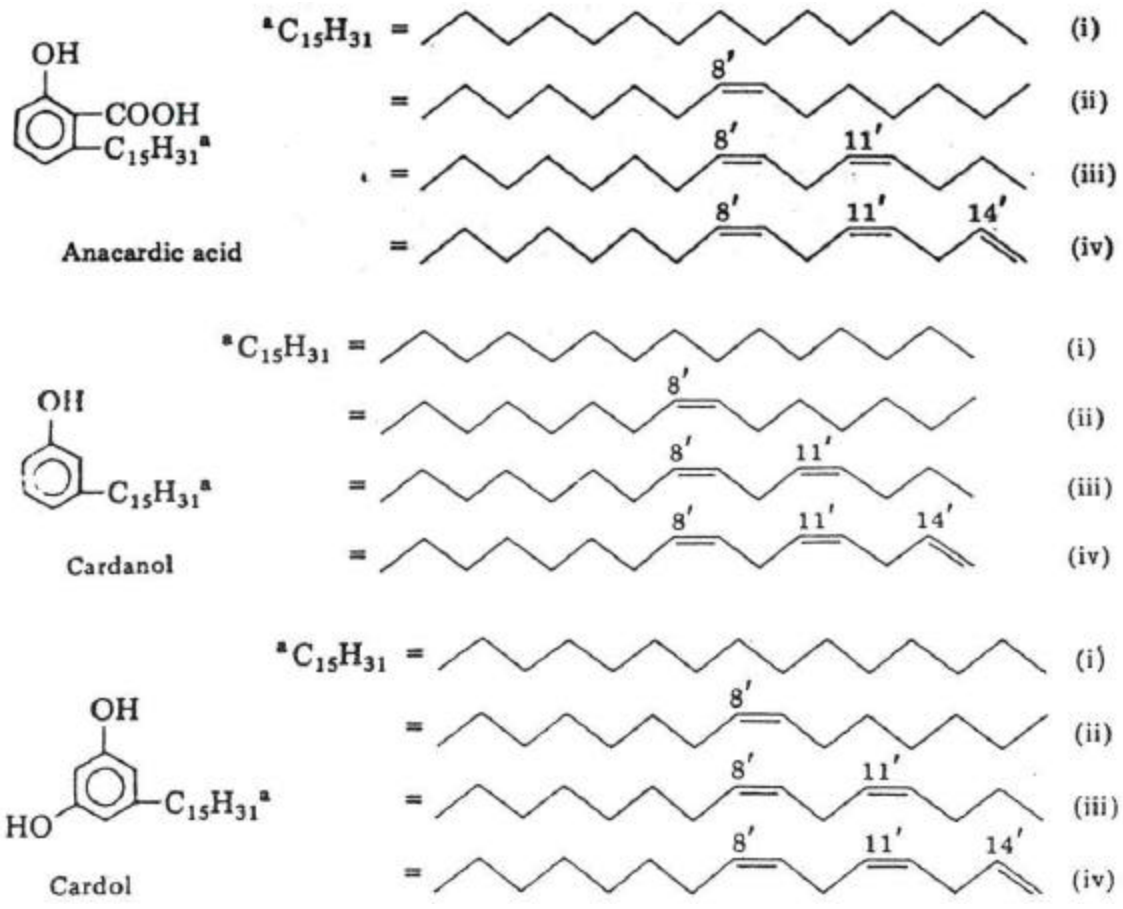
CNSL is traditionally obtained as a by-product during the process of removing the cashew kernel from the nut. The processes used are mainly hot-oil and roasting in which the CNSL oozes out from the shell.

The cashew tree is cultivated globally in tropical areas such as East Africa, South and Central America and the Far East. The world availability of CNSL is in the region of 50,000 tons/year.

1.1 Composition

Natural (i.e. cold, solvent extracted) CNSL contains approximately 70% anacardic acid (Fig 1), 18% cardol, and 5% cardanol, with the remainder being made up of other phenols and less polar substances. As can be seen in Figure 1, anacardic acid, cardanol and cardol consist of mixtures of components having various degrees of unsaturation in the alkyl side-chain.

Figure 1: Structures of Anacardic acid, Cardanol and Cardol



In technical (i.e. heat extracted) CNSL, the heating process leads to decarboxylation of the anacardic acid to form cardanol. Typically, the composition of technical CNSL is approximately 52% cardanol, 10% cardol, 30% polymeric material, with the remainder being made up of other substances.

The technical CNSL is often further processed by distillation at reduced pressure to remove the polymeric material. The composition of distilled technical CNSL is approximately 78% cardanol, 8% cardol, 2% polymeric material and the remainder other substances.

Table 1 summarises the composition of typical batches of technical and distilled technical grades of CNSL.

Table 1: Composition of typical batches of Technical and Distilled CNSL

	Cardanol	Cardanol monoene	Cardanol diene	Cardanol triene	Cardol diene	Cardol triene	Polymer	Unidentified
T-CNSL	0.06	17.10	10.78	24.42	2.36	7.50	30.6	5.83
D-CNSL	-	25.9	16.2	35.8	2.04	5.90	2.5	9.70
AT-CNSL	0.09	24.7	15.6	35.3	3.41	10.8	-	8.42

T-CNSL = Technical grade, D-CNSL = Distilled grade, AT-CNSL = Technical grade component percentages adjusted for removal of polymer.

1.2 Commercial Applications

CNSL resins have been used extensively in the manufacture of friction-resistant components in applications such as brake and clutch linings. These resins are used as binders for friction ingredients and also as friction ingredients themselves in the form of fine dusts obtained from the completely cured resins.

CNSL-aldehyde condensation products and CNSL-based phenolic resins are used in applications such as surface coatings, adhesives, varnishes and paints. Various polyamines synthesised from CNSL or cardanol are used as curing agents for epoxy resins.

CNSL and its derivatives have been used as antioxidants, plasticisers and processing aids for rubber compounds and modifiers for plastic materials. Resins based on the reaction products of cardanol phenol and formaldehyde are used to improve the resistance of rubber articles to cracking and ozone. CNSL, cardanol and cardol are all used to provide oxidative resistance to sulfur-cured natural rubber products. Cardanol, CNSL or sulfurated CNSL is added to rubber gum stock or nitrile rubber to improve the processability, mechanical properties and resistance to crack and cut properties of the vulcanisates.

A number of products based on CNSL are used as antioxidants, stabilisers and demulsifiers for petroleum products. Metal xanthates of partially hydrogenated, sulfurised cardanol is used to lower the pour point of lubricating oils as well as acting as antioxidant and anticorrosive properties. Soluble metal derivatives of CNSL are used to improve the resistance to oxidation and sludge formation of lubricating oils. Oxidised CNSL and its derivatives are used as demulsifying agents for water in oil type petroleum emulsions.

1.3 Worker / consumer exposure

Only large industrial manufacturers use CNSL. There are no direct consumer applications and therefore no direct sales to the general public. The most likely source of consumer exposure to

CNSL is through contact with contaminated nuts, although reports of adverse effects arising from such contact appear to be rare.

Exposure of workers to CNSL during production is most likely to occur during removal of the kernels from the nuts, after processing to remove the CNSL, especially in countries where the shelling has not been mechanised. Exposure to CNSL can lead to sensitisation and dermatitis. Workers in these countries are given some protection to exposure through the use of barrier creams.

Workers involved in the further processing the CNSL to manufacture commercial products are likely to have minimal exposure to the CNSL as it is expected that good industrial hygiene practices will be followed and personal protective equipment worn to minimise exposure.

2. Rationale for Selection of Compounds for testing

Distilled Technical CNSL has been selected as the most suitable substance for testing to fulfil the requirements of the HPV Challenge Program. This is because it is possible to obtain distilled CNSL to a more consistent specification than the Technical CNSL and the distilled grades are becoming more important industrially than the crude technical grade material. It is also believed that since all of the substances in CNSL are based on phenols having various degrees of unsaturation in the side chain, they meet the EPA's criterion of using the 'family approach', thus the toxicological properties of the Distilled CNSL will be representative of the properties of the Technical CNSL.

3. Review of Existing Data and Development of Test Plan

Cardolite Corporation has undertaken a comprehensive evaluation of all relevant data on the SIDS endpoints of concern for Cashew Nut Shell Liquid.

The availability of the data on the specific SIDS endpoints is summarized in Table 2. Table 2 also shows data gaps that will be filled by additional testing.

Table 2: Available Adequate Data and Proposed Testing on Cashew Nut Shell Liquid*

CAS No. 8007-24-7	Information Available?	GLP	OECD Study?	Other Study?	Estimation Method?	Acceptable?	SIDS Testing required?
	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Physicochemical							
Partition Coefficient (Kow)	N	-	-	-	-	-	Y
Water solubility	N	-	-	-	-	-	Y
Environmental Fate & Pathway							
Biodegradation	Y	Y	Y	-	-	Y	N
Ecotoxicology							
Acute Fish	Y	-	-	-	Y	Y	N
Acute Daphnia	Y	-	-	-	Y	Y	N
Acute Algae	Y	-	-	-	Y	Y	N
Toxicology							
Acute Oral	N	-	-	-	-	-	Y
Repeat Dose toxicity	N	-	-	-	-	-	Y

Genetic toxicity – Gene mutation	Y	Y	Y	-	-	-	N
Genetic toxicity – Chromosome aberration	Y	Y	Y	-	-	-	N
Reproductive toxicity	N	-	-	-	-	-	Y
Developmental toxicity/teratogenicity	N	-	-	-	-	-	Y

- No testing will be conducted for melting point, boiling point, vapor pressure, photodegradation, hydrolysis or transport and distribution between environmental compartments.

A. Evaluation of Existing Physicochemical Data and Proposed Testing

The basic physicochemical data required in the SIDS battery includes melting point, boiling point, vapor pressure, partition coefficient (Kow) and water solubility.

Cashew nut shell liquid (CNSL) meets the criteria for a Class 2 substance – it is a natural product that contains a number of chemical species and is of variable composition depending on its source and is, therefore, difficult to characterize and cannot be represented by a single chemical structural diagram. Due to this 'complex mixture' characteristic of CNSL, some physical property measurements do not give definitive results because the methodology used to determine these properties will fractionate or partition the substance into various components. Since the methodology will alter the actual sample composition, the results are likely to be erroneous or difficult to interpret.

1. Melting Point

Melting point will not be determined, as the substance is a liquid under ambient conditions.

2. Boiling Point

A boiling point at ambient pressure has no significance, as the substance will be subject to thermal polymerization and decomposition before boiling. Accordingly, measurement of this property is inappropriate for this substance.

3. Vapor Pressure

Estimation of the vapor pressure for the 2 main chemical components, and their analogs, of CNSL using EPIWIN v 3.04[(1)] predicts the vapor pressure to be less than $2x10^{-5}$ Pa (i.e. negligible) at ambient temperatures. Experimental measurement is inappropriate.

4. Water solubility

Assuming adequate analytical sensitivity can be achieved, the water solubility of CNSL using OECD Method 105 will be determined, although estimation using EPIWIN predicts the solubility of cardanol, cardol and their structural analogs to be between 1 - $7x10^{-3}$ mg/L.

5. Partition Coefficient

The partition coefficient (i.e. Kow) for CNSL will be determined using OECD Method 107. It is likely that more than one Kow value, rather than a single value, will be generated when this endpoint is determined. This outcome reflects the complex nature of Class 2 mixtures.

Summary of Physicochemical Properties Testing: The water solubility (OECD method 105) and partition coefficient (OECD 107) of CNSL will be determined. Tests for melting point, boiling point and vapor pressure are inapplicable to these substances.

B. Evaluation of Existing Environmental Fate Data and Proposed Testing

The fate or behaviour of a chemical in the environment is determined by the reaction rates for the most important transformation (degradation) processes. The basic environmental fate data covered by the HPV Program include biodegradation, stability in water (hydrolysis as a function of pH), photodegradation and transport and distribution between environmental compartments.

1. Biodegradation

Biodegradability provides a measure for the potential of compounds to be degraded by microorganisms. Depending on the nature of the test material, several standard test methods are available to assess potential biodegradability.

Distilled CNSL has been shown to be biodegradable when tested using OECD Method 302D (96% degradation after 28 days) in a GLP study.

2. Hydrolysis

Hydrolysis as a function of pH is used to assess the stability of a substance in water. Hydrolysis is a reaction in which a water molecule (or hydroxide ion) substitutes for another atom or group of atoms present in an organic molecule. None of the major components of CNSL contain a functional group that would be susceptible to hydrolysis. Therefore, hydrolysis need not be measured.

In addition, low water solubility often limits the ability to determine hydrolysis as a function of pH. Estimation of the water solubility of the 2 main chemical components, and their analogs, of CNSL using EPIWIN v 3.04 predicts the solubility to be in the region of $1x10^{-3}$ mg/L. Therefore, these materials are expected to be stable in water and it would be unnecessary to attempt to measure the products of hydrolysis.

3. Photodegradation

Due to the extremely low vapor pressure under ambient conditions, there is essentially no opportunity for CNSL to enter the atmosphere. Thus, photodegradation is irrelevant. Further, as photodegradation is estimated as part of the model used to calculate the transport and distribution between environmental compartments, difficulties in providing the correct inputs to the model mean that it is not possible to derive a meaningful value for this endpoint.

4. Transport and Distribution between Environmental Compartments

The transport and distribution between environmental compartments is intended to determine the ability of a chemical to move or partition in the environment. The determination of this property requires the use of various models (e.g., level III model from the Canadian Environment Modeling Centre Trent University). For Class 2 substances such as CNSL, the required inputs to the model are either not available or not feasible to determine including molecular mass, reaction half-life estimates for air, water, soil, sediment, aerosols, suspended sediment and aquatic biota. In addition, while the partition coefficient is also required and can be determined, the multiple Kow values typically derived for such substances are a consequence of sample fractionation and reflect

various components in the mixture and are not representative of the mixture itself. Consequently, due to the inability to provide usable inputs to the required model, no determination of transportation and distribution between environmental compartments will be undertaken for CNSL.

Summary of Environmental Fate Testing: Biodegradation data exists for distilled CNSL. Photodegradation, hydrolysis and transport and distribution between environmental compartments are not applicable to this substance.

C. Evaluation of Existing Ecotoxicity Data and Proposed Testing

The basic ecotoxicity data that are part of the HPV program include acute toxicity to fish, daphnia and algae. Predicted values for the 2 main chemical components, and their analogs, of CNSL have been obtained using ECOSAR v 0.99e[(2)]. These values predict that CNSL will be toxic in the aquatic environment, therefore it is unnecessary to generate experimental data. This is further supported by the predicted very low solubility of cardanol, cardol and their structural analogs, which are in the region of $1 - 7 \times 10^{-3}$ mg/L, making analytical monitoring of the tests very difficult and making the tests themselves unlikely to determine any more useful or accurate estimates of toxicity than the estimations.

Summary of Ecotoxicity Testing: The 2 main components of CNSL, and their analogs, are predicted to be acutely toxic to fish (LC_{50} (96h) < 11×10^{-3} mg/l), daphnia (LC_{50} (48h) < 66×10^{-3} mg/l) and algae (EC_{50} (96h) < 1×10^{-3} mg/l).

D. Evaluation of Existing Human Health Effects Data and Proposed Testing

1. Acute Oral Toxicity

Acute oral toxicity investigates the effects of a single exposure to a relatively high dose of a substance. This test is conducted by administering the test material to animals (typically rats or mice) in a single gavage dose. Harmonized EPA testing guidelines (August 1998) set the limit dose for acute oral toxicity studies at 2000 mg/kg body weight. A compound that shows no effects at the limit dose is considered essentially non-toxic.

The acute oral toxicity of CNSL will be determined using OECD 425.

2. Repeat Dose Toxicity

Subchronic repeat dose toxicity studies are designed to evaluate the effect of repeated exposure to a chemical over a significant period of the life span of an animal. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of between 28 – 90 days. The HPV program calls for a repeat dose test of at lest 28 days. Repeat dose studies are designed to assess systemic toxicity, but the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

The repeat dose toxicity of CNSL, combined with the reproductive/developmental toxicity screening test will be determined using OECD 422.

3. Genotoxicity

Genetic testing is conducted to determine the effects of substances on genetic material (i.e. DNA and chromosomes). The gene, which is composed of DNA, is the simplest functional genetic unit. Mutations of genes can occur spontaneously or as a consequence of exposure to chemicals or

radiation. Genetic mutations are commonly measured in bacterial and mammalian cells, and the HPV program calls for completing both types of tests.

Distilled CNSL has been tested for potential genotoxicity in the Ames Salmonella assay (strains TA1535, TA1537, TA1538, TA98 and TA100), an in vitro chromosome aberration test in human lymphocytes, and an in vitro HGPRT forward mutation assay using a Chinese Hamster Ovary cell line. None of these test systems showed any indication of genotoxicity. All three studies were conducted under GLP.

4. Reproductive and Developmental Toxicity

Reproductive toxicity includes any adverse effect on fertility and reproduction, including effects on gonadal function, mating behaviour, conception and parturition. Developmental toxicity is any adverse effect induced during the period of fetal development, including structural abnormalities, altered growth and post-partum development of the offspring.

The toxicity to reproduction aspect of the HPV Challenge Program can be met by conducting a reproductive/developmental toxicity screening test or adding a reproductive/developmental screening test to the repeat dose study (OECD 421 or 422, respectively).

As there is no repeat dose toxicity data for CNSL, the substance will be tested for repeat dose toxicity combined with the reproductive/developmental toxicity screening test according to OECD 422.

5. Skin Sensitisation

This non-SIDS endpoint has been evaluated using distilled CNSL in a Guinea pig maximisation test (OECD 406). The test substance produced a 70% (14/20) sensitisation rate and was classified as a strong sensitiser.

6. Oestrogenic Activity

This non-SIDS endpoint has been evaluated using two grades of distilled CNSL in a recombinant yeast screen assay. The two distillates showed no oestrogenic activity under the conditions of the test.

Summary of Human Health Effects Testing: The acute oral toxicity of CNSL will be determined using OECD 425. The repeat dose toxicity combined with the reproductive and developmental toxicity will be evaluated using OECD 422. Distilled CNSL has been tested and found negative in three in vitro genotoxicity assays, therefore no additional testing for this endpoint will be undertaken. Distilled CNSL has been shown to be a strong skin sensitiser in guinea pigs and to have no oestrogenic activity when tested in a recombinant yeast screen assay.

4. Evaluation of Data for Quality and Acceptability

The collected data were reviewed for quality and acceptability following the general US EPA guidance (3) and the systematic approach described by Klimisch et al (4). These methods include consideration of the reliability, relevance and adequacy of the data in evaluating their usefulness for hazard assessment purposes. This scoring system was only applied to ecotoxicology and human health endpoint studies per EPA recommendation (5). The codification described by Klimisch specifies four categories of reliability for describing data adequacy. These are:

(1) Reliable without restriction: Includes studies or data complying with Good Laboratory Practice (GLP) procedures, or with valid and/or internationally accepted testing guidelines, or in which the test parameters are documented and comparable to these guidelines.

(2) Reliable with Restrictions: Includes studies or data in which test parameters are documented but vary slightly from testing guidelines.

(3) Not Reliable: Includes studies or data in which there are interferences, or that use non-relevant organisms or exposure routes, or which were carried out using unacceptable methods, or where documentation is insufficient.

(4) Not Assignable: Includes studies or data in which insufficient detail is reported to assign a rating, e.g. listed in abstracts or secondary literature.

7. References

1. EPIWIN v3.04. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

2. ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

3. USEPA (1998). Guidance for Meeting the SIDS Requirements (The SIDS Guide). Guidance for the HPV Challenge Program. Dated 11/2/98.

4. Klimisch, H.-J., et al (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regul. Toxicol. Pharmacol. 25:1-5

5. USEPA (1999). Determining the Adequacy of Existing Data. Guidance for the HPV Challenge Program. Draft dated 2/10/99.

AR201-13793B

HUMAN HEALTH ENDPOINTS (NON SIDS)
OESTROGENIC ACTIVITY

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NX 4708 (distilled cashew nut shell liquid)
Source: Cardanol Chemicals N.V., Lot No. LT-0481

METHOD

- **Method:** Routledge and Sumpter (1996)
- **Test Type:** Recombinant Yeast Screen A ssay
- **System of testing**: Non bacterial
- **GLP:** Yes
- **Year:** 1999
- **Species/Strain:** Saccharomyces cerevisiae, recombinant strain containing the human oestrogen receptor (hER) and the reporter gene *lac -Z* (encoding for the enzyme ß-galactosidase).
- **Concentrations tested:** 0.049, 0.098, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 mg/l
- **Statistical Methods:** None

Remarks:

- **Test Design:**
  - **Number of replicates:** 2
  - **Frequency of dosing:** Single
  - **Positive control:** 17β-estradiol, Bisphenol A
- **Solvent:** Ethanol

RESULTS

- **Result:** Negative

Remarks: None

CONCLUSIONS

Remarks:

No oestrogenic activity was observed at all concentrations tested.

In accordance with current regulatory guidelines for the environmental classification of chemicals it was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/l.

Care should be taken in the interpretation of these results, as a negative result in this in vivo study does not necessarily indicate that the material will not have an oestrogenic effect in the environment.

Bisphenol A was determined to be 3500 times less potent that 17β-estradiol.

The response of the recombinant yeast screen to both of the positive control materials was comparable to published results thereby confirming the suitability of the innoculum and culture conditions.

REFERENCES (Free Text)

SafePharm Laboratories Ltd., Cardolite NX4708: Assessment of oestrogenic activity using a recombinant yeast screen assay, Report No. 814/005, June 1999

Routledge EJ and Sumpter JP, 1996, Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen, Environmental Toxicology and Chemistry 15: 241-248

OTHER

- **Last Changed:** 20 May 2002
- **Order number for sorting:** 3

HUMAN HEALTH ENDPOINTS (NON SIDS)
OESTROGENIC ACTIVITY

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 700 (distilled cashew nut shell liquid)
Source: Cardanol Chemicals N.V., Lot No. GT457

METHOD

- **Method:** Routledge and Sumpter (1996)
- **Test Type:** Recombinant Yeast Screen Assay
- **System of testing**: Non bacterial
- **GLP:** Yes
- **Year:** 1999
- **Species/Strain:** Saccharomyces cerevisiae, recombinant strain containing the human oestrogen receptor (hER) and the reporter gene *lac-Z* (encoding for the enzyme ß-galactosidase).
- **Concentrations tested:** 0.049, 0.098, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 mg/l
- **Statistical Methods:** None

Remarks:

- **Test Design:**

  - **Number of replicates:** 2
  - **Frequency of dosing:** Single
  - **Positive control:** 17β-estradiol, Bisphenol A

- **Solvent:** Ethanol

RESULTS

- **Result:** Negative

Remarks: None

CONCLUSIONS

Remarks:

No oestrogenic activity was observed at all concentrations tested.

In accordance with current regulatory guidelines for the environmental classification of chemicals it was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/l.

Care should be taken in the interpretation of these results, as a negative result in this in vivo study does not necessarily indicate that the material will not have an oestrogenic effect in the environment.

Bisphenol A was determined to be 3500 times less potent that 17β-estradiol.

The response of the recombinant yeast screen to both of the positive control materials was comparable to published results thereby confirming the suitability of the innoculum and culture conditions.

REFERENCES (Free Text)

SafePharm Laboratories Ltd., Cardolite NC700: Assessment of oestrogenic activity using a recombinant yeast screen assay, Report No. 814/004, June 1999

Routledge EJ and Sumpter JP, 1996, Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen, Environmental Toxicology and Chemistry 15: 241-248

OTHER

- **Last Changed:** 15 May 2002
- **Order number for sorting:** 2

HUMAN HEALTH ENDPOINTS (NON SIDS)
SKIN SENSITIZATION

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 700 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: EQ-1

METHOD

- **Method:** OECD 406, 'Skin Sensitisation'.
- **Species/strain:** Albino Dunkin Hartley guinea pigs.
- **Concentration**
  - **Intradermal induction:** 1% w/v in liquid paraffin
    1% w/v in a mixture of Freund's Complete Adjuvant plus distilled water (1:1)
  - **Topical induction:** 25% v/v in liquid paraffin
  - **Topical challenge:** 5% and 2% v/v in liquid paraffin
- **No of animals/sex/dose:** 20 females/dose
- **Vehicle:** Liquid Paraffin BP
- **GLP:** Yes
- **Year:** 1996

Remarks: None

RESULTS

- Sensitization rate: 14/20 (70%) sensitised
- Result: Positive

Remarks:

Skin reactions observed after intradermal induction: Well-defined erythema (grade 2) was commonly noted at the intradermal injection sites at the 24-hour observation. Incidents of moderate to severe erythema were also noted at this time. Well-defined erythema persisted at all intradermal injection sites at the 48-hour observation.

Skin reactions observed after topical induction: Very slight or well-defined erythema (grades 1 or 2) with or without very slight oedema (grade 1), was commonly noted at the topical induction sites at the 1-hour observation. Incidents of fissuring of the skin, or bleeding were also noted at this time. The bleeding was probably caused by self-inflicted scratching of the skin.

Skin reactions observed after topical challenge with 5% v/v Cardolite NG700: Very slight or well-defined erythema (grade 1 or 2) was noted at the challenge sites of eleven animals at the 24-hour observation. Very slight oedema (grade 1) was also noted at five of these sites at this observation. Very slight erythema (grade 1) was noted at the challenge sites of 14 animals at the 48-hour observation, with very slight oedema (grade 1) at two of these sites.

Desquamation was seen at the challenge sites of seven animals. No evidence of erythema or oedema was seen at the 72-hour observation, although the presence of desquamation precluded evaluation of erythema at the challenge sites of none animals at this time.

Skin reactions observed after topical challenge with 2% v/v Cardolite NC700: Very slight or well-defined erythema (grade 1 or 2) was noted at the challenge sited of six animals at the 24-hour observation. Very slight oedema (grade 1) was also noted at one of these sites at this observation. Very slight erythema (grade 1) was noted at the challenge sited of five animals at the 48-hour observation. No skin reactions were noted at the challenge sites of two of these animals at the 24-hour observation. Desquamation was noted at one challenge site at the 48-hour observation. Very slight erythema (grade 1) persisted at the challenge site of one animal at the 72-hour observation. Desquamation was noted at the challenge sites of three animals at this time.

Clinical observations: All animals showed an expected gain in bodyweight over the study period. No signs of ill-health were noted in any animal.

CONCLUSIONS

Remarks: Cardolite NC-700 produced a 70% (14/20) sensitisation rate in this study and was classified as a strong as a strong sensitiser.

REFERENCES (Free Text)

SafePharm Laboratories Ltd., Determination of the skin sensitisation potential of Cardolite NC-700 and assessment of cross-sensitisation potential with poison ivy oil, Cardolite NC-513, Cardolite NC-514, Cardolite NC-541 and Cardolite NC0558, Report No. 661/010, February 1996

OTHER

- Last Changed: 20 May 2002
- Order number for sorting: 1

Remarks: None

ECOTOXICITY ENDPOINTS
11. TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

TEST SUBSTANCE

- **Identity:** Cashew Nut Shell Liquid

Remarks: Test substance: Cardanol (CAS No. 37330-39-5), Cardol (CAS No. 57486-25-6)

METHOD

- **Method:** Calculation using ECOSAR v.0.99e
- **Type:** N/A
- **GLP:** No
- **Year:** 2002
- **Species:** Algae

Remarks: None

RESULTS

- **Unit:** mg/L
- **EC_{50} at 96 hours:** 0.00011 – 0.00034 (Cardanol)
  0.00031 – 0.00096 (Cardol)

Remarks: The predicted EC_{50} values vary with the degree of unsaturation in the alkyl side chain of Cardanol and Cardol as follows:

	EC_{50}, mg/L			
	unsaturated	monoene	diene	triene
Cardanol	0.00011	0.00017	0.00026	0.00034
Cardol	0.00031	0.00048	0.00072	0.00096

CONCLUSIONS

Remarks:

Estimation using ECOSAR v0.99e predict Cardanol and Cardol, the two major components of distilled and technical grade Cashew Nut Shell Liquid, to be toxic to algae.

DATA QUALITY

Reliabilities: 4, Not Assignable

Remarks:

Estimation using ECOSAR v.0.99e

REFERENCES (Free Text)

ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

OTHER

- Last Changed: 24 April 2002
- Order number for sorting: 1

Remarks:

HUMAN HEALTH ENDPOINTS
15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: LP-2

METHOD

- **Method:** OECD 473
- **Test Type:** Chromosomal aberration test
- **System of testing:** Non bacterial
- **GLP:** Yes
- **Year:** 1995
- **Species/Strain:** Human Lymphocytes
- **Metabolic activation:** S9-mix, Rat liver cells, Aroclor induced, 1 ml
- **Concentrations tested:** Expt. 1 (20h harvest): 0, 6.25, 12.5, 25 µg/ml (-S9)
  0, 3.125, 6.25, 12.5 µg/ml (+S9)
  Expt. 2 (20h harvest): 12.5, 25, 37.5 µg/ml (-S9)
  0.78, 1.56, 3.125, µg/ml (+S9)
  Expt. 2 (44h harvest): 25 µg/ml (-S9)
  3.125 µg/ml (+S9)

- **Statistical Methods:** Fisher's Exact test or Chi-squared test

Remarks:

- **Test Design**

  - **Number of replicates:** 2
  - **Positive control:** Ethyl methanesulphonate (EMS) (-S9), cyclophosphamide (+S9)
  - **Negative control:** Solvent vehicle

- **Solvent:** Dimethylsulfoxide

RESULTS

- **Result:** Negative
- **Cytotoxic concentration**

  - **With metabolic activation:** 12.5 µg/ml
  - **Without metabolic activation:** >25 µg/ml

- **Genotoxic effects**
  - **With metabolic activation:** None
  - **Without metabolic activation:** None

- **Statistical results:** The test material did not induce a significant increase in the frequency of cells with chromosome aberrations or polyploid cells in either the presence or absence of a liver enzyme metabolizing system.

Experiment 1: Harvest Time 20 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	1	0	0	0	1	1	1	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	0	0	1	0	0	0	1	1	1	1
			(0.0)	(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
6.25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
12.5 µg/ml	A	100	2	1	0	0	0	0	3	1	3	1
	B	100	0	0	0	1	0	0	1	1	1	1
	Total	200	2	1	0	1	0	0	4	2	4	2
			(1.0)	(0.5)	(0.0)	(0.5)	(0.0)	(0.0)	(2.0)	(1.0)	(2.0)	(1.0)
25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	1	1	0	0	0	0	2	1	2	1
	Total	200	2	1	0	0	0	0	3	1	3	1
			(1.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(0.5)	(1.5)	(0.5)
Positive Control EMS 500 µg/ml	A	50	31	14	7	2	1	0	55	24	33	21
	B	50	13	18	8	2	0	0	41	28	29	24
	Total	100	44	32	15	4	1	0	96	52	62***	45***
			(44.0)	(32.0)	(15.0)	(4.0)	(1.0)	(0.0)	(96.0)	(52.0)	(62.0)	(45.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells *** represents $p \leq 0.001$

Experiment 1: Harvest Time 20 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	0	1	0	0	0	0	1	1	1	1
			(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
1.56 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	0	1	0	0	0	0	1	1	1	1
	Total	200	0	1	0	0	0	0	1	1	1	1
			(0.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.5)	(0.5)	(0.5)
3.125 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	1	0	0	0	0	0	1	0	1	0
	Total	200	2	0	0	0	0	0	2	0	2	0

			(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.0)
6.25 µg/ml	A	100	0	0	0	0	0	0	0	0	0	0
	B	100	4	0	0	0	0	0	4	0	4	0
	Total	200	4	0	0	0	0	0	4	0	4	0
			(2.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(2.0)	(0.0)	(2.0)	(0.0)
Positive Control CP 25 µg/ml	A	100	4	0	0	1	0	0	5	1	5	1
	B	100	1	2	0	2	0	0	5	4	4	3
	Total	200	5	2	0	3	0	0	10	5	9**	4
			(2.5)	(1.0)	(0.0)	(1.5)	(0.0)	(0.0)	(5.0)	(2.5)	(4.5)	(2.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

** represents $p \leq 0.01$

Experiment 2: Harvest Time 20 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	2	0	0	0	0	0	2	0	2	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	2	0	0	0	0	0	2	0	2	0
			(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.0)
12.5 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
25 µg/ml	A	100	1	1	0	0	0	0	2	1	2	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	1	0	0	0	0	2	1	2	1
			(0.5)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
37.5 µg/ml	A	100	1	1	0	0	0	0	2	1	2	1
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	1	0	0	0	0	2	1	2	1
			(0.5)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
Positive Control EMS 500 µg/ml	A	100	6	9	4	0	0	0	19	13	13	11
	B	100	16	17	2	1	0	0	36	20	26	15
	Total	200	22	26	6	1	0	0	55	33	39***	26***
			(11.0)	(13.0)	(3.0)	(0.5)	(0.0)	(0.0)	(27.5)	(16.5)	(19.5)	(13.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

*** represents $p \leq 0.001$

Experiment 2: Harvest Time 20 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	1	1	1	0	0	0	3	2	3	2
	B	100	0	1	0	0	0	0	1	1	1	1

	Total	200	1	2	1	0	0	0	4	3	4	3
			(0.5)	(1.0)	(0.5)	(0.0)	(0.0)	(0.0)	(2.0)	(1.5)	(2.0)	(1.5)
0.78	A	100	0	0	0	0	0	0	0	0	0	0
µg/ml	B	100	0	3	0	0	0	0	3	3	3	3
	Total	200	0	3	0	0	0	0	3	3	3	3
			(0.0)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
1.56	A	100	1	0	0	0	0	0	1	0	1	0
µg/ml	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)
3.125	A	100	0	0	0	0	0	0	0	0	0	0
µg/ml	B	100	1	0	0	0	1	0	2	1	2	1
	Total	200	1	0	0	0	1	0	2	1	2	1
			(0.5)	(0.0)	(0.0)	(0.0)	(1.0)	(0.0)	(1.0)	(0.5)	(1.0)	(0.5)
Positive	A	100	5	4	0	1	0	0	10	5	9	5
Control	B	100	6	0	2	1	0	0	9	3	8	3
CP	Total	200	11	4	2	2	0	0	19	8	17**	8
25			(5.5)	(2.0)	(1.0)	(1.0)	(0.0)	(0.0)	(9.5)	(4.0)	(8.5)	(4.0)
µg/ml												

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells ** represents $p \leq 0.01$

Experiment 2: Harvest Time 44 hours, without metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	3	0	0	0	0	3	3	3	3
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	0	3	0	0	0	0	3	3	3	3
			(0.0)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
25 µg/ml	A	100	1	0	0	0	0	0	1	0	1	0
	B	100	0	0	0	0	0	0	0	0	0	0
	Total	200	1	0	0	0	0	0	1	0	1	0
			(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.5)	(0.0)	(0.5)	(0.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

Experiment 2: Harvest Time 44 hours, with metabolic activation

Treatment Group	Replicate ID	No. Cells Scored	Total Gaps	Chromatid		Chromosome		Others	Total Aberrations		Aberrant Cells	
				Breaks	Exchanges	Breaks	Exchanges	X	(+Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	100	0	0	0	1	0	0	1	1	1	1
	B	100	0	1	0	1	0	0	2	2	2	2
	Total	200	0	1	0	2	0	0	3	3	3	3
			(0.0)	(0.5)	(0.0)	(1.0)	(0.0)	(0.0)	(1.5)	(1.5)	(1.5)	(1.5)
25 µg/ml	A	100	2	1	0	1	0	0	4	2	4	2
	B	100	1	0	0	0	0	0	1	0	1	0
	Total	200	3	1	0	1	0	0	5	2	5	2

			(1.5)	(0.5)	(0.0)	(0.5)	(0.0)	(0.0)	(2.5)	(1.0)	(2.5)	(1.0)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells

Experiment 1: Mean Frequency of Polyploid Cells (%)

Dose Level µg/ml	20 Hours	
	Without S9	With S9
0	0.0	0.0
1.56	-	0.5
3.125	-	0.0
6.25	0.0	0.0
12.5	0.0	-
25	0.0	-
EMS 500	0.0	-
CP 25	-	0.0

Experiment 2: Mean Frequency of Polyploid Cells (%)

Dose Level µg/ml	Without S9		Dose Level µg/ml	With S9	
	20 hours	44 hours		20 hours	44 hours
0	0.0	0.5	0	0.0	1.0
12.5	0.5	-	0.78	0.0	-
25	0.0	0.5	1.56	1.0	-
37.5	0.0	-	3.125	1.0	0.0
EMS 500	0.0	-	CP 25	0.5	-

Remarks:

Experiment 1: Mitotic Index (20-hour harvest)

Dose Level µg/ml	Without S9				With S9			
	A	B	Mean	% of Control	A	B	Mean	% of Control
0	5.80	6.25	6.03	100	3.10	2.40	2.75	100
0.78					-	-	-	-
1.56	-	-	-	-	-	-	-	-
3.125	-	-	-	-	3.60	3.60	3.60	131
6.25	4.90	7.80	6.35	105	1.15	2.25	1.70	62
12.5	6.70	6.50	6.60	109	0.85	0.55	0.70	25
25	8.30	4.30	6.30	104	-	-	-	-
50	NM	NM	-	-				
EMS 500	3.40	4.30	3.85	64				
CP 25	-	-	-	-	1.40	2.45	1.93	70

- = not assessed NM = no scorable metaphases

Experiment 2: Mitotic Index (20-hour harvest)

Dose	Without S9	With S9

Level µg/ml	A	B	Mean	% of Control	A	B	Mean	% of Control
0	8.55	7.90	8.23	100	3.00	3.25	3.13	100
0.39					-	-	-	-
0.78					1.80	3.35	2.58	82
1.56					2.50	2.80	2.65	85
3.125	-	-	-	-	1.35	1.90	1.63	52
6.25	7.20	6.75	6.98	85	0.45	0.45	0.45	14
9.38					NM	NM	-	-
12.5	7.75	9.45	8.60	104				
25	6.00	9.45	7.73	94				
37.5	3.25	3.65	3.45	42				
50	NM	NM	NM	-				
EMS 500	4.70	7.95	4.83	59	-	-	-	-
CP 25	-	-	-	-	1.60	1.45	1.53	49

- = not assessed NM = no scorable metaphases

CONCLUSIONS

Remarks: The substance was found to be non-clastogenic under the conditions of the test.

DATA QUALITY

- **Reliabilities**: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: Chromosome Aberration Test in Human Lymphocytes In Vitro, Report No. 814/002, 1995

Scott, D., Et al, Metaphase chromosome aberration assays in vitro. In: Kirkland, D.J., Basic mutagenicity tests: UKEMS recommended procedures. Report. Part 1 revised. Cambridge University Press, 1990:62-84

OTHER

- **Last Changed:** 25 April 2002
- **Order number for sorting:** 2

Remarks:

HUMAN HEALTH ENDPOINTS
15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: LP-2

METHOD

- **Method:** OECD 471
- **Test Type:** Reverse Mutation Assay (Ames Test)
- **System of testing:** Bacterial
- **GLP:** Yes
- **Year:** 1995
- **Species/Strain:** Salmonella typhimurium (TA1535, TA1537, TA1538, TA98 & TA100)
- **Metabolic activation:** S9-mix, Rat liver cells, 0.5 ml, Aroclor induced
- **Concentrations tested:** 50, 150, 500, 1500, 5000 µg/plate (±S9)
- **Statistical Methods:** Dunnett's method of linear regression

Remarks:

- **Test Design**

  - **Number of replicates:** 3
  - **Positive controls:** N-ethyl-N'-nitro-N-nitrosoguanidine (-S9, TA100 & TA1535)
    9-aminoacridine (-S9, TA 1537)
    4-nitro-o-phenylenediamine (-S9, TA1538)
    4-nitroquinoline-1-oxide (-S9, TA98)
    2-aminoanthracene (+S9, TA98, TA100, TA1535, TA1537 & TA1538)
  - **Negative control:** Solvent vehicle

- **Solvent:** Acetone

RESULTS

- **Result:** Negative
- **Cytotoxic concentration**

  - **With metabolic activation:** >5000 µg/plate
  - **Without metabolic activation:** >5000 µg/plate

- **Genotoxic effects**
  - **With metabolic activation:** None
  - **Without metabolic activation:** None

- **Statistical results:** No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation.

Experiment 1 – Without Metabolic Activation

Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	115 117 (110) 97 11.0	28 25 (21) 9 10.2	34 25 (25) 17 8.5	36 30 (30) 25 5.5	10 17 (15) 18 4.4
50	149 118 (131) 127 15.9	20 19 (19) 18 1.0	17 27 (23) 24 5.1	17 24 (21) 23 3.8	19 18 (18) 18 0.6
150	118 120 (120) 121 1.5	12 10 (12) 15 2.5	9 28 (16) 11 10.4	24 17 (18) 14 5.1	15 18 (17) 18 1.7
500	121 115 (116) 111 5.0	17 20 (16) 12 4.0	13 30 (18) 12 10.1	17 33 (25) 25 8.0	10 12 (12) 14 2.0
1500	115p 107p (117) 128p 10.6	8p 22p (16) 17p 7.1	15p 17p (15) 14p 1.5	22p 25p (27) 34p 6.2	13p 10p (12) 13p 1.7
5000	122p 85p (106) 111p 19.0	7p 15p (10) 8p 4.4	25p 15p (19) 18p 5.1	29p 22p (23) 17p 6.0	12p 19p (16) 18p 3.8
Positive Control	ENNG	ENNG	4NOPD	4NQO	9AA
Concentration (µg/plate)	3	5	5	0.2	80
Number of colonies per plate	670 933 (729) 583 182.2	198 213 (224) 260 32.3	470 474 (479) 494 12.9	168 152 (156) 149 10.2	76 208 (152) 172 68.2

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive controls: ENNG (N-ethyl-N'-nitro-N-nitroguanidine), 4NOPD (4-nitro-o-phenylenediamine), 4NQO (4-nitroquinloine-1-oxide), 9AA (9-aminoacridine)

Experiment 1 – With Metabolic Activation					
Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537

0	131 108 (113) 101 15.7	19 17 (17) 15 2.0	39 35 (33) 24 7.8	24 38 (32) 35 7.4	17 18 (16) 14 2.1
50	118 129 (125) 127 5.9	18 13 (15) 15 2.5	34 24 (32) 39 7.6	29 34 (33) 35 3.2	17 13 (16) 17 2.3
150	121 111 (117) 120 5.5	13 19 (16) 17 3.1	32 33 (33) 33 0.6	33 33 (35) 39 3.5	10 15 (15) 19 4.5
500	111 143 (115) 91 26.2	10 15 (15) 19 4.5	18 35 (29) 34 9.5	35 38 (33) 27 5.7	12 15 (12) 10 2.5
1500	98p 133p (114) 112p 17.6	20p 17p (19) 20p 1.7	25p 28p (27) 28p 1.7	28p 28p (30) 33p 2.9	13p 18p (15) 13p 2.9
5000	128p 112p (117) 112p 9.2	12p 17p (14) 13p 2.6	30p 18p (24) 25p 6.0	29p 28p (29) 30p 1.0	14p 19p (17) 17p 2.5
Positive Control	2AA	2AA	2AA	2AA	2AA
Concentration (µg/plate)	1	2	0.5	0.5	2
Number of colonies per plate	1332 1615(1382) 1200 212.0	64 69 (66) 64 2.9	353 323 (361) 408 43.1	219 453 (329) 315 117.6	194 252 (232) 250 32.9

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive control: 2AA (2-aminoanthracene)

Experiment 2 – Without Metabolic Activation					
Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	107 132 (116) 110 13.7	14 18 (17) 19 2.6	29 22 (21) 12 8.5	17 23 (21) 24 3.8	19 19 (17) 13 3.5

50	149 133 (140) 138 8.2	12 18 (21) 32 10.3	41 22 (29) 23 10.7	34 30 (32) 0	10 20 (14) 13 5.1
150	133 118 (129) 137 10.0	18 24 (24) 29 5.5	10 22 (17) 19 6.2	35 33 (32) 28 3.6	14 10 (12) 12 2.0
500	134 139 (131) 121 9.3	13 13 (15) 20 4.0	20 14 (21) 30 8.1	23 34 (25) 19 7.8	13 19 (19) 24 5.5
1500	117p 117p (109) 92p 14.4	12p 10p (12) 14p 2.0	22p 23p (21) 18p	17p 20p (26) 40p 12.5	18p 12p (15) 14p 3.1
5000	107p 121p (108) 95p 13.0	10p 25p (19) 23p 8.1	19p 19p (20) 23p 2.3	20p 24p (28) 39p 10.0	14p 12p (12) 10p 2.0
Positive Control	ENNG	ENNG	4NOPD	4NQO	9AA
Concentration (µg/plate)	3	5	5	0.2	80
Number of colonies per plate	916 711 (711) 740 110.9	514 504 (498) 477 19.1	406 499 (455) 459 46.7	177 203 (196) 208 16.6	638 656 (589) 474 100.3

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive controls: ENNG (N-ethyl-N'-nitro-N-nitroguanidine), 4NOPD (4-nitro-o-phenylenediamine), 4NQO (4-nitroquinloine-1-oxide), 9AA (9-aminoacridine)

Experiment 2 – With Metabolic Activation					
Test Substance Concentration (µg/plate)	Number of revertants (Number of colonies per plate)				
	Base-pair substitution type		Frameshift type		
	TA100	TA1535	TA1538	TA98	TA1537
0	137 139 (131) 117 12.2	25 20 (20) 15 5.0	35 28 (34) 39 5.6	24 28 (32) 44 10.6	22 18 (18) 13 4.5
50	133 138 (128) 112 13.8	24 22 (21) 18 3.1	31 33 (32) 32 1.0	38 33 (34) 32 3.2	19 24 (19) 13 5.5

150	108 120 (115) 118 6.4	23 30 (25) 22 4.4	25 35 (34) 43 9.0	29 36 (33) 35 3.8	14 22 (17) 14 4.6
500	122 142 (125) 110 16.2	23 24 (23) 23 0.6	23 30 (26) 25 3.6	28 24 (32) 44 10.6	13 13 (16) 22 5.2
1500 5000	129p 120p (123) 121p 4.9 128p 170p (135) 106p 32.5	13p 15p (19) 30p 9.3 18p 20p (18) 15p 2.5	25p 22p (25) 28p 3.0 32p 17p (29) 38p 10.8	28p 13p (26) 36p 11.7 36p 35p (36) 36p 0.6	17p 17p (16) 14p 1.7 15p 15p (15) 14p 0.6
Positive Control	2AA	2AA	2AA	2AA	2AA
Concentration (µg/plate)	1	2	0.5	0.5	2
Number of colonies per plate	1398 1553(140 6) 1268 142.7	102 139 (114) 102 21.4	276 256 (273) 286 15.3	159 293 (243) 278 73.4	255 250 (254) 258 4.0

Key to Table: 'number of revertants' – observed values and average values (in parentheses) are shown at each dose. Figures immediately below average values refer to standard deviation. The letter 'p' following a number indicates precipitation was observed.

Positive Control: 2AA (2-aminoanthracene)

Remarks: A precipitate was observed at and above 1500 µg/plate, however this did not interfere with the scoring of revertant colonies.

CONCLUSIONS

Remarks: The substance was found to be non-mutagenic under the conditions of the test.

DATA QUALITY

- **Reliabilities**: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: Reverse Mutation Assay 'Ames Test' Using Salmonella Typhimurium, Report No. 814/001, 1995

Kirkland, D.J., (Ed), Statistical Evaluation of Mutagenicity Test Data, UKEMS Sub-committee on Guidelines for Mutagenicity Testing, Report - Part III (1989), Cambridge University Press

OTHER

- **Last Changed:** 25 April 2002
- **Order number for sorting:** 1

Remarks:

ENVIRONMENTAL FATE ELEMENTS AND PATHWAYS
9. BIODEGRADATION

TEST SUBSTANCE

- **Identity:** Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation. Lot No.: LP-2

METHOD

- **Method:** OECD Method 302D
- **Test Type:** aerobic
- **GLP:** Yes
- **Year:** 1993
- **Contact time:** 28 (days)
- **Innoculum:** activated sludge

Remarks:

- **Innoculum:** Fresh activated sludge from a municipal biological sewage treatment plant. 30 mg suspended solids/l of test medium.
- **Concentration of test chemical:** 6.01 – 6.39 mg, direct addition
- **Temperature of incubation:** 20°C
- **Dosing procedure:** Test substance weighed on a piece of glass to an amount of about 20 mg ThOD (or COD) and added directly to the test flask.
- **Sampling frequency:** 0,7,14,21 & 28 days
- **Controls:** Sodium acetate used as positive control, innoculum used as blank.
- **Analytical method used to measure biodegradation:** The COD of the poorly soluble substance was determined in a variation of ISO Method 6060 (closed system with a pressure equaliser / Kelkenberg method, Z.f. Wasser und Abwasserforschung (1975) 146). Oxygen determination was performed using an oxygen electrode (WTW;FRG; Microprocessor oximeter OXI 2000 with electrode model TriOxmatic EO 200).
- **Method of calculating measured concentrations:** Arithmetic mean

RESULTS

- **Degradation % after time:** 96% after 28 days
- **Results:** Readily biodegradable
- **Kinetic:**

Day	% Degradation	
	Sample	Positive control
7	46	75
14	72	86
21	86	91
28	96	97

- **Breakdown products:** Not determined

Remarks: None

CONCLUSIONS

Remarks:

According to the author of the study, based on the data (i.e. 96% degradation after 28 days) Cardolite NC-511 can be regarded as very highly biodegradable.

DATA QUALITY

- **Reliabilities:** 1, Reliable without restriction

Remarks: Study conducted under GLP to recognised test method by Henkel KGaA

REFERENCES

Henkel KGaA, Cardolite NC-511 Ultimate biodegradability in the BODIS-Test, Report No. RE930104, 1993

OTHER

Last Changed: 23 April 2002
Order number for sorting: 1

Remarks:

The test method used was based on the closed bottle test (OECD test method 302D) and the RDA-Blok test, previously published (Blok, J., A Repetitive Die Away (RDA) Test Combining Several Biodegradability Test Procedures, Int. Biodeterior. Bull., 15 (1979) 57-63) and ring-tested by the OECD (1988 ring test on ready biodegradability).

ECOTOXICITY ENDPOINTS
10. ACUTE TOXICITY TO FISH

TEST SUBSTANCE

- **Identity:** Cashew Nut Shell Liquid

Remarks: Test substance: Cardanol (CAS No. 37330-39-5), Cardol (CAS No. 57486-25-6)

METHOD

- **Method:** Calculation using ECOSAR v.0.99e
- **Type:** N/A
- **GLP:** No
- **Year:** 2002
- **Species:** Fish

Remarks: None

RESULTS

- **Unit:** mg/L
- **LC_{50} at 96 hours:** 0.002 – 0.005 (Cardanol)
  0.005 – 0.011 (Cardol)

Remarks: The predicted LC_{50} values vary with the degree of unsaturation in the alkyl side chain of Cardanol and Cardol as follows:

	LC_{50}, mg/L			
	unsaturated	monoene	diene	triene
Cardanol	0.002	0.003	0.004	0.005
Cardol	0.005	0.007	0.009	0.011

CONCLUSIONS

Remarks:

Estimation using ECOSAR v0.99e predict Cardanol and Cardol, the two major components of distilled and technical grade Cashew Nut Shell Liquid, to be toxic to fish.

DATA QUALITY

- **Reliabilities:** 4, Not Assignable

Remarks:

Estimation using ECOSAR v0.99e

REFERENCES

ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

OTHER

- Last Changed: 24 April 2002
- Order number for sorting: 1

Remarks:

ECOTOXICITY ENDPOINTS
12. TOXICITY TO AQUATIC INVERTIBRATES (E.G., DAPHNIA)

TEST SUBSTANCE

- **Identity:** Cashew Nut Shell Liquid

Remarks: Test substance: Cardanol (CAS No. 37330-39-5), Cardol (CAS No. 57486-25-6)

METHOD

- **Method:** Calculation using ECOSAR v.0.99e
- **Type:** N/A
- **GLP:** No
- **Year:** 2002
- **Species:** Daphnia

Remarks: None

RESULTS

- **Unit:** mg/L
- **LC_{50} at 48 hours:** 0.024 – 0.040 (Cardanol)
  0.039 – 0.066 (Cardol)

Remarks: The predicted LC_{50} values vary with the degree of unsaturation in the alkyl side chain of cardanol and cardol as follows:

	LC_{50}, mg/L			
	unsaturated	monoene	diene	triene
Cardanol	0.024	0.029	0.035	0.040
Cardol	0.039	0.048	0.058	0.066

CONCLUSIONS

Remarks:

Estimation using ECOSAR v0.99e predicts Cardanol and Cardol, the two major components of distilled and technical grade Cashew Nut Shell Liquid, to be toxic to Daphnia.

DATA QUALITY

- **Reliabilities:** 4, Not Assignable

Remarks:

Estimation using ECOSAR v.0.99e

REFERENCES

ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

OTHER

- Last Changed: 24 April 2002
- Order number for sorting: 1

Remarks:

HUMAN HEALTH ENDPOINTS
15. GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

TEST SUBSTANCE

- Cashew Nutshell Liquid

Remarks: Test substance: Cardolite NC 511 (distilled cashew nut shell liquid)
Source: Cardolite Corporation, Lot No.: LP-2

METHOD

- **Method:** OECD 476
- **Test Type:** Forward Mutation Assay
- **System of testing:** Non bacterial
- **GLP:** Yes
- **Year:** 1996
- **Species/Strain:** Chinese Hamster Ovary CHO-KI BH4
- **Metabolic activation:** S9-mix, Rat liver cells, Aroclor induced
- **Concentrations tested:** Expt. 1: 0, 0.75, 1.5, 3, 6, 12 µg/ml (-S9)
  0, 1.5, 3, 6, 12, 18 µg/ml (+S9)
  Expt. 2: 0, 0.75, 1.5, 3, 6, 9 µg/ml (-S9)
  0, 3, 6, 12, 18, 24 µg/ml (+S9)

- **Statistical Methods:** Cochran-Armitage test for trend analysis, Fisher-Irwin exact test for group comparisons for proportions.

Remarks:

- **Test Design**

  - **Number of replicates:** 2
  - **Positive control:** Ethyl methanesulphonate (EMS) (-S9), 3-methylcholanthrene (3-MC) (+S9)
  - **Negative control:** Solvent vehicle

- **Solvent:** Dimethylsulfoxide

RESULTS

- **Result:** Negative
- **Cytotoxic concentration**

  - **With metabolic activation:** 47.19 µg/ml
  - **Without metabolic activation:** 47.19 µg/ml

- **Genotoxic effects**
  - **With metabolic activation:** None
  - **Without metabolic activation:** None

- **Statistical results:** The test material did not induce significant or dose-related increases in mutant frequency per survivor in either the presence or absence of metabolic activation in either of the two experiments.

Summary of Results:

Experiment 1:

Dose Level µg/ml	Without S9		Mean MFS	Dose Level µg/ml	With S9		Mean MFS
	A	B			A	B	
0	3.4	0.7	2.05	0	3.5	3.3	3.4
0.75	1.4	-	1.4	1.5	2.9	0.7	1.80
1.5	2.0	0.0	1.00	3.0	0.6	1.4	1.00
3	0.0	0.0	0.0	6.0	2.9	0.0	1.45
6	0.0	0.0	0.0	12	1.4	6.3	3.85
12	0.0	6.3	3.15	18	0.7	8.6	4.65
EMS 200	154.5	189.9	172.20	24	-	-	-
				3-MC 4	238.8	285.9	262.35

MFS = 6-TG resistant mutants/10^6 viable cells

Experiment 2:

Dose Level µg/ml	Without S9		Mean MFS	Dose Level µg/ml	With S9		Mean MFS
	A	B			A	B	
0	0.0	0.6	0.30	0	8.1	0.8	4.45
0.75	0.4	7.6	4.00	3	1.3	0.0	0.65
1.5	3.2	0.9	2.05	6	0.9	0.0	0.45
3	0.6	6.2	3.40	2	0.0	0.0	0.00
6	0.5	1.7	1.10	18	0.0	0.0	0.00
9	0.6	0.0	0.30	24	TOXIC		
EMS 200	158.3	149.1	153.70				
				3-MC 4	284.6	278.1	281.35

MFS = 6-TG resistant mutants/10^6 viable cells

Remarks:

CONCLUSIONS

Remarks: The test material was found to be non-mutagenic to CHO cells at the HGPRT locus under the conditions of this test.

DATA QUALITY

- **Reliabilities**: 1, Reliable without restriction

Remarks: Study conducted under GLP to OECD test guideline by SafePharm Laboratories Ltd.

REFERENCES (Free Text)

Safepharm Laboratories Ltd., Cardolite NC 511: CHO HGPRT Forward Mutation Assay, Report No. 814/003, 1996

Cole, J., et al, (1990): Gene Mutation in Cultured Mammalian Cells. In 'Basic Mutagenicity Tests: UKEMS Recommended Procedures', (ed D.J. Kirkland), Cambridge University Press, New York,

OTHER

- **Last Changed:** 26 April 2002
- **Order number for sorting:** 3

Remarks: